February 14, 1901.

A. B. KEMPE, M.A., Treasurer and Vice-President, in the Chair.

A List of the Presents received was laid on the table, and thanks ordered for them.

The following Papers were read:—

I. "Some Additional Notes on the Orientation of Greek Temples, being the Result of a Journey to Greece and Sicily, in April and May, 1900." By F. C. PENROSE, F.R.S.

II. "The Transmission of the *Trypanosoma Evansi* by Horse Flies, and other Experiments pointing to the Probable Identity of Surra of India and Nagana or Tsetse-fly Disease of Africa." By Dr. LEONARD ROGERS. Communicated by Major D. BRUCE, R.A.M.C., F.R.S.

III. "On the Influence of Ozone on the Vitality of some Pathogenic and other Bacteria." By Dr. A. RANSOME, F.R.S., and A. G. R. FOULERTON.

IV. "On the Functions of the Bile as a Solvent." By B. MOORE and W. H. PARKER. Communicated by Professor SCHÄFER, F.R.S.

V. "On the Application of the Kinetic Theory of Gases to the Electric, Magnetic, and Optical Properties of Diatomic Gases." By G. W. WALKER. Communicated by Professor RÜCKER, Sec. R.S.

VI. "Heredity, Differentiation, and other Conceptions of Biology: A Consideration of Professor Karl Pearson's Paper 'On the Principle of Homotyposis.'" By W. BATESON, F.R.S.

"On the Influence of Ozone on the Vitality of some Pathogenic and other Bacteria." By ARTHUR RANSOME, M.D., F.R.C.P., F.R.S., and ALEXANDER G. R. FOULERTON, F.R.C.S. Received January 12,—Read February 14, 1901.

The influence of ozone on the vitality of bacteria is a matter which has received the attention of several investigators. But, on reviewing the records of the results which have been arrived at, it is obvious that such results have not always been consistent.

We determined, therefore, to investigate this question anew, in the hope of being able to come to a definite conclusion. The matter seemed to us to be one of considerable importance, since if ozone were possessed of the bactericidal properties which have been attributed to it by more than one investigator, the gas might prove of much value in solving one of the most unsatisfactory problems which have to be dealt with in the practice of modern sanitation, that is to say, the disinfection of rooms after the occurrence of infectious disease. Ozone can now be conveniently produced in large quantities, and, if efficient, would be admirably adapted to effect the purpose in view.

The question of the bactericidal action of ozone was especially brought into prominence by the classical work of Downes and Blunt, embodied in communications made to this Society in 1877 and 1878.* Working with impure cultures of bacteria, these investigators showed that direct sunlight in the presence of atmospheric air was capable in some cases of preventing in greater or less degree, or in other cases of absolutely inhibiting, the growth of the particular bacteria experimented with; and that not only might growth be inhibited, but that the bacteria themselves might be actually destroyed. Downes and Blunt further showed that so far as the destruction of bacteria is concerned the blue and violet rays of the spectrum are more effective than the red rays, that the interposition of a layer of water is sufficient to protect the bacteria to a certain extent, and that direct sunlight acting *in vacuo* may fail to destroy sporing bacteria.

Whilst this work of Downes and Blunt has been fully confirmed and amplified in certain directions by the work of others, no satisfactory explanation has yet been arrived at as to exactly how it is that bacteria are destroyed under these conditions. The explanation that the result is a direct effect of the sun's rays—of heat—has been shown to be untenable; and it has therefore been assumed that the destruction is effected by chemical rather than by physical action; that it results from an active oxidation of the substance of the bacteria by ozone, produced by the action of sunlight on atmospheric air. Others have regarded peroxide of hydrogen as the active agent.

Amongst the experiments which have been carried out in order to test this assumed bactericidal action of ozone, we may particularly mention those of Chapuis,† Sonntag‡, and Ohlmüller.§ Chapuis filtered air through cotton wool, and then exposed plugs of the wool with the contained bacteria to the action of ozone. The plugs were afterwards incubated in a nutrient wort solution, which remained sterile. Control plugs of the wool which had not been subjected to the action

* 'Roy. Soc. Proc.,' vol. 26, p. 488; vol. 28, p. 199; vol. 40, p. 14.

† 'Bulletin de la Société Chimique,' 1881, Tome 35, p. 290.

‡ 'Centralblatt für Bakteriologie.' Erste Abteilung, Band 8, p. 778, 1890.

§ 'Arbeiten a. d. Kaiserl. Gesundheitsamte,' 1892, Band 8, p. 229.

of the ozone, gave rise to a free growth of bacteria, when incubated in the same medium. Sonntag and Ohlmüller's experiments, on the other hand, seemed to show that ozone in the dry state had little or no action on bacteria, but was capable of destroying them when passed through water containing them. Thus *B. anthracis*, suspended in distilled water, was destroyed after air containing 9·6 millegrammes of ozone per litre had been passed through the mixture for ten minutes. A sporing culture of the same bacillus was killed by passing air containing 15·2 milligrammes of ozone per litre through the water for ten minutes. If, however, organic matter, such as blood serum, were added to the water the results were different; and it seemed that under these latter conditions the most part of the ozone was expended in oxidation of the dead organic matter present, whilst the bacteria were little if at all affected.

Our experiments were planned with the view of ascertaining whether ozone applied in large quantities, either in a mixture with atmospheric air or with pure oxygen, has in reality a destructive influence on bacterial life, and especially whether it has any such influence under conditions which would enable it to be used for practical purposes of disinfection.

The experiments have included the testing of the action of ozone, (1) on the vitality of certain pathogenic and saprophytic bacteria, and (2) on the virulence of one pathogenic species. For the purposes of the latter test, we decided to test the action of the gas on *B. tuberculosis*, an organism which is known to be readily affected by the direct action of ordinary chemical agents, and one which numerous experiments would lead us to believe is very susceptible to the action of direct sunlight (Koch,* Ransome and Delepine,† and Jousset).‡

Experiment I.—In our first experiment, culture tubes with "sloped" surface of nutrient agar or gelatin were inoculated with various bacteria; a mixture of atmospheric air and ozone was passed continuously over the inoculated surface for a period of at least four hours, commencing twenty-four hours after the tubes were inoculated. The tubes were then incubated at appropriate temperatures, and the result compared with that obtained in control tubes which had been inoculated from the same stock cultures at the same time.

In detail the following was the procedure carried out:—The culture tubes were of the ordinary 15 × 2 cm. size, into the sides of which short pieces of 0·75 cm. calibre glass tubing had been blown in such a way that they opened into the lumen of the culture tubes about 3 cm. from the bottom and just above the lower level of the sloped nutrient

* 'Ueber bacteriologische Forschung.' Introductory Address, Tenth International Medical Congress, August 4, 1890.

† 'Roy. Soc. Proc.,' vol. 56.

‡ 'Comptes Rendus de la Société de Biologie,' 1900, Tome 52, p. 884.

surface, and allowed the ozonised air to escape after passing over the bacteria. The culture tubes were closed at the upper end by a piece of cork through which passed a short length of the 0·75 cm. tubing, which formed the inlet for the ozonised air.

The inlet and outlet tubes were loosely plugged with cotton wool, and by means of them and short lengths of india-rubber tubing the culture tubes could be connected up in series, and sterile ozonised air drawn over the inoculated surfaces.

Such culture tubes were inoculated with the following bacteria:—

Glycerin-agar	tubes	(Nos. 1 to 6) with	*Bacillus tuberculosis.*
,,	,,	(Nos. 7 and 8) ,,	*Bacillus mallei.*
Nutrient-agar	,,	(Nos. 9 and 10) ,,	*Bacillus diphtheriæ.*
,,	,,	(Nos. 11 ,, 12) ,,	*Bacillus anthracis* (sporing).
Nutrient-gelatin	,,	(Nos. 13 ,, 14) ,,	*Bacillus typhosus.*
,,	,,	(Nos. 15 ,, 16) ,,	*Micrococcus melitensis.*
,,	,,	(Nos. 17 ,, 18) ,,	*Micrococcus candicans.*

The tubes were then arranged in two series, those numbered 1 to 12 being connected up in one series and those numbered 13 to 18 in another. The two series of tubes were then placed in a room of about 900 cubic feet capacity and ozone was generated in the air of the room by means of four small "ozonisers," a 3-inch spark Ruhmkorff coil and an accumulator battery being used. The "ozonisers" were kept working for four hours, during the whole of which time ozonised air was slowly aspirated through the tubes. At the end of four hours the arrangement of the tubes was altered; a fresh series, including those numbered 3 to 12 and 15 to 18, being connected up, and pure oxygen charged with ozone was forced through the tubes for a period of thirty minutes. During this half-hour ozonised air was still being drawn through tubes 13 and 14. The culture tubes were then incubated, Nos. 1 to 12 at 37° C., and Nos. 13 to 18 at 22° C., the respective control tubes being incubated with them. The result of the experiment was that in the case of two out of the seven species tested, there seemed to have been some slight retardation of growth as the result of the exposure to the ozone; that is to say, in the case of one or both of the duplicate tubes containing *Bacillus mallei* and *Bacillus diphtheriæ*, the growth of the experimental cultures seemed at first to be rather slower than it was in the corresponding control tubes. But at the end of eight days' incubation all difference between the experimental and control tubes had disappeared, and growth was equal in both sets; and in further experiments this effect was not obvious. In the case of the other five species not the slightest effect could be observed as the result of the exposure. This experiment was carried out under conditions which, although they might approximate to those which would prevail in the actual use of ozone as an aerial disinfectant, were not adapted to

test the action of ozone on bacteria, apart from an important disturbing factor. The bacteria were submitted to the action of ozone in the presence of a large amount of dead organic matter, and it was quite conceivable that such an amount of the ozone might have been decomposed in the oxidation of the dead organic matter that but little had been left to exert any action on the living bacteria.*

Experiment II.—In this experiment we endeavoured to test the action of ozone on the bacteria in the absence—so far as we could ensure the condition—of dead organic matter. The same culture tubes were used, but instead of inoculating agar or gelatin nutrient surfaces we inoculated small blocks of plaster of Paris from stock cultures of the various bacteria tested. These plaster of Paris blocks when inoculated were placed in the culture tubes, and the inlet and outlet tubes were plugged with fine Italian asbestos fibre instead of with cotton wool. And instead of passing the same current of ozone over a series of tubes in succession, we connected each tube separately with a main feeding pipe with lateral branches, the respective tubes being held in contact by pieces of india-rubber tubing. Thus each culture tube had a fresh supply of ozone. Ozone was generated as before, and passed over blocks inoculated from stock cultures of the following :—

1. *Staphylococcus pyogenes aureus.*
2. *Streptococcus pyogenes.*
3. *Micrococcus melitensis.*
4. *Bacillus mallei.*
5. *Bacillus diphtheriæ.*
6. *Bacillus anthracis* (from sporing culture).
7. *Bacillus typhosus.*
8. *Bacillus coli communis.*
9. *Bacillus pyocyaneus.*
10. *Bacillus pneumoniæ* (Friedlander).
11. *Bacillus prodigiosus.*
12. *Saccharomyces albicans.*

Duplicate tubes were inoculated with each organism, and a continuous current of air was pumped over the ozoniser, which was enclosed within a glass cylinder connected with the main feeding tube, and then through the culture tubes for a period of thirty minutes. The actual amount of ozone used was not estimated, but iodide of potassium and starch paper held over the outlet tubes gave a positive reaction within sixty seconds of the commencement of the experiment. The small plaster of Paris blocks were then shaken up in tubes containing 3 c.c. of nutrient broth, from the broth tubes loopfuls were transferred to other media, and the growth obtained after incubation compared with the growth on control tubes.

The results obtained on incubating the sub-cultures made it evident that none of the bacteria had been affected by the ozone in such a way as to impair either their capability of growth, or, in the case of the two

* We are indebted to Mr. Bridge, chemist, of Bournemouth for assistance in the working of the ozonising apparatus used in carrying out this experiment.

chromogenic bacteria, their function of pigment production. The pathogenic action of a broth sub-culture of *B. mallei*, after the ozonisation, was tested by intra-peritoneal inoculation of a male guinea-pig; an ordinary infection with characteristic lesions followed, the animal dying within forty-eight hours.

Experiment III.—We now decided to subject the bacteria to a rather more severe test than had been involved in the two preceding experiments. The ozone was produced by passing oxygen under pressure from a cylinder over a powerful "ozoniser," enclosed within a glass cylinder, and then into the main feeding tube, as in the previous experiment. The current used was an alternating one direct from the street main. Small pieces of porcelain were, after inoculation with the following bacteria, placed in the culture tubes:—

1. *Sarcina ventriculi.*
2. *Micrococcus melitensis.*
3. *Micrococcus candicans.*
4. *Bacillus mallei.*
5. *Bacillus diphtheriæ.*
6. *Bacillus anthracis* (from twenty-four hour old culture in broth, non-sporing).
7. *Bacillus anthracis* (from old sporing culture on potato).
8. *Bacillus typhosus.*
9. *Bacillus coli communis.*
10. *Bacillus pyocyaneus.*
11. *Bacillus pneumoniæ.*
12. *Bacillus prodigiosus.*

Duplicate tubes of each species were used for the experiment, the first attempt to carry out which resulted in failure, owing to the action of the ozone on the pieces of india-rubber tubing by which the branches of the main feeding tube and the inlets into the culture tubes were held in contact. Before the mixture of ozone and oxygen had been passed into the series of culture tubes for ninety seconds, every piece of india-rubber tubing was cut through, as if with a knife. The joints were, therefore, made with pieces of bored cork, and the experiment repeated. The mixture of ozone and oxygen was passed through the tubes at the rate of 1·5 litre per minute for a period of thirty minutes; the yield of ozone, as estimated by titration with $\frac{N}{10}$ iodide solution, amounted to 0·072 gramme per minute. The percentage amount of ozone was therefore about 2·4 by volume. At the end of thirty minutes the pieces of porcelain were dropped into tubes of nutrient broth and incubated. On comparison with the various controls it was obvious that the ozone had not affected the bacteria in such a way as to impair either their capability for growth, or, in the case of the chromogenic organisms, their power of producing pigment. The broth sub-culture of *B. anthracis* (non-sporing) after forty-eight hours' incubation at 37° C. was tested on a white mouse, and proved to be of normal

virulence; 0·25 c.c. of the broth culture, injected into the peritoneal sac, killing the animal within twenty-four hours in typical fashion.*

Experiment IV.—Although it seemed to have been conclusively proved by the experiments of Ohlmüller, already referred to, that ozone was capable of considerable bactericidal action when the organisms were suspended in certain fluids, we determined to carry out a single experiment, using milk as the medium. We used milk because we considered that it would, as containing a large quantity of organic matter, test the bactericidal action of the gas severely.

Five flasks, each containing 125 c.c. of milk, were prepared as follows:—

Flask 1 contained sterilised milk which had been inoculated with a culture of *B. anthracis* (sporing).

Flask 2 contained sterilised milk which had been inoculated with a non-sporing culture of *B. anthracis*.

Flask 3 contained ordinary fresh unsterilised milk, to which a quantity of a broth culture of *B. prodigiosus* had been added.

Flask 4 contained ordinary fresh unsterilised milk.

Flask 5 contained a sample of commercial "sterilised" milk which had "gone bad" owing to the presence in pure culture of an anaerobic, sporing, butyric acid forming bacillus.

A current of oxygen containing the same proportion of ozone as that used in Experiment III was passed through the milk in each of the flasks for a period of twenty minutes at the rate of 1·5 litres per minute. Loopfuls of milk were then taken from each flask, transferred to various culture media, and incubated under both aerobic and anaerobic conditions; the flasks with the bulk of the milk still remaining in them were also incubated.

In the result, it was found that the contents of flasks 1, 2 and 5 were sterile of bacteria. The milk used for flasks 3 and 4 was taken from the same sample, and on incubation of the sub-cultures after ozonisation a growth of a mould-fungus was obtained from each flask; from flask 3 a very free growth of the mould was obtained, but neither *B. prodigiosus* or any other bacterium; from flask 4 a few colonies of a coccus were obtained in both aerobic and anaerobic cultures in addition to the mould which was present apparently in less quantity in the contents of flask 4 than it was in the contents of flask 3. In the case of the sub-cultures from flask 3, the growth of the mould was very rapid, and soon covered the surface of the medium, and so possibly checked the growth of the coccus which appeared on the sub-cultures from flask 4, in which the mould growth was less abundant.

A loopful of the milk used for flasks 3 and 4, taken before ozonisation and smeared over nutrient agar, gave, on incubation at 22° C.,

* We are indebted to Mr. Wood Smith, F.I.C., for assistance in the working of the ozonising apparatus used in this experiment.

a large number of colonies of different bacteria; and it was apparent that the exposure to ozone had resulted in the destruction of a large majority of these, although complete sterilisation was not obtained as in the case of flasks 1, 2, and 5.

At the end of the experiment, the milk in flasks 1, 2, 3, and 4, although not changed in appearance, had acquired an extremely disagreeable taste and smell, which was in all probability at least partly due to the development of fatty acids. It seemed therefore possible that in the case of these milks, not only might the ozone have had a directly injurious action on the bacteria, but it might also have affected them indirectly by producing from the natural milk various bodies which might themselves also have to be considered as factors in the experiment.

The milk in flask 5 was in a late stage of decomposition and possessed of a most offensive odour; it was noticed that the offensiveness of this milk was considerably reduced after the passage of the ozone.

Experiment V.—Our next experiment was made in order to ascertain whether ozone had any influence on the virulence as apart from the mere vitality of *B. tuberculosis*, and was carried out in the following way:—Sputum rich in the specific bacillus was smeared over strips of filter-paper. These strips were then dried, and afterwards exposed for varying periods to the action of highly-ozonised air. The exposure was ensured by pinning out the strips on a board, which was hung about 6 feet from the same ozonising apparatus as that used in Experiment I, and in the same room. The apparatus was set at work two hours before the exposure of the sputum was commenced, and was continued without intermission throughout the experiment. When the exposure was commenced the air of the room was so highly charged with ozone as to be extremely unpleasant, and not respirable by anyone for more than a few minutes at a time. After undergoing exposures of the several durations given in the table below, the strips of infected paper were moistened, stretched out on glass, and the surface which had been smeared with the sputum was scraped off lightly with the edge of a knife. The scraping from each strip was collected in a cubic centimetre of sterilised normal saline solution, and doses of 0·2 c.c. of the emulsions thus obtained were injected under the skin of the inguinal fold in guinea-pigs. As controls, other guinea-pigs were similarly inoculated with some of the crude sputum, and also with the scrapings from an infected strip of paper which had not been previously ozonised. Fourteen animals in all were inoculated; the following table gives their weights and the nature of the emulsion used for each:—

Animal.	Weight.	Inoculated with—
	grammes.	
Guinea-pig I..	500	Small quantity of crude sputum.
,, II..	470	,, ,, ,,
,, III..	390	Emulsion from filter-paper, not ozonised.
,, IV..	339	,, ,, ,, ,,
,, V .	420	,, ,, ,, ozonised ½ hour.
,, VI..	436	,, ,, ,, ,, ½ ,,
,, VII..	450	,, ,, ,, ,, 1 ,,
,, VIII..	455	,, ,, ,, ,, 1 ,,
,, IX..	390	,, ,, ,, ,, 2 hours.
,, X .	450	,, ,, ,, ,, 2 ,,
,, XI..	370	,, ,, ,, ,, 4 ,,
,, XII..	370	,, ,, ,, ,, 4 ,,
,, XIII..	410	,, ,, ,, ,, 8 ,,
,, XIV..	370	,, ,, ,, ,, 8 ,,

The various animals were either allowed to die naturally or were killed with chloroform after definite signs of tubercular infection had developed. And it may at once be said that a severe infection occurred in all the animals; there was not the least indication that the ozonisation had exerted any effect whatever on the virulence of the bacilli. As examples, we may mention the following animals:—Guinea-pig No. I died on the twentieth day after inoculation, with a caseous abscess in the flank, infected mesenteric glands, and tubercles in the spleen; guinea-pig No. II was killed on the twenty-second day after inoculation, and was found to be in a similar condition; guinea-pig No. XI died on the twenty-second, and guinea-pig No. XIV on the twenty-third day, both being again in a similar stage. The presence of the specific bacillus in one or other of the internal lesions was proved in the case of every animal on the list.

Conclusions.

Our experiments have made it clear that ozone in the dry state, and in such strength as we used it, has no appreciable action on the vitality of the various bacteria experimented with, and, so far, our results are in accordance with those of Sonntag and Ohlmüller. Nor did a prolonged exposure to the action of ozone diminish in any way the pathogenic virulence of *B. tuberculosis* in sputum, as shown by Experiment V. Single experiments would also tend to show that ozone can have little, if any, effect on the pathogenic virulence of *B. mallei* and *B. anthracis*.

On the other hand, Experiment IV would appear to confirm the conclusion arrived at by Ohlmüller as to the bactericidal property of ozone when passed through a fluid medium containing bacteria in suspension.

A comparison of the inactivity of ozone as a disinfectant in the dry state with its action in the presence of water suggests a superficial resemblance with other gases, such as chlorine and sulphur dioxide. In the absence of further experiment, however, it would not be possible to press the analogy too closely.

In the dry state, and under the conditions in which it occurs in nature, ozone, then, is not capable of any injurious action on bacteria so far as can be judged from our experiments; and we conclude that any purifying action which ozone may have in the economy of nature is due to the direct chemical oxidation of putrescible organic matter, and that it does not in any way hinder the action of bacteria, which latter are, indeed, in their own way, working towards the same end as the ozone itself in resolving dead organic matter to simpler non-putrescible substances.

"On the Functions of the Bile as a Solvent." By BENJAMIN MOORE and WILLIAM H. PARKER. Communicated by Professor SCHÄFER, F.R.S. Received January 24,—Read February 14, 1901.

The purpose of the biliary secretion and the uses of that fluid in digestion and otherwise have furnished much material for discussion to the physiological chemist, and the discussion has given rise to many ingenious but widely different theories.

The bile, unlike all the other digestive fluids which are secreted into the alimentary canal, has no specific action upon any of the three classes of food-stuffs. It contains small amounts of cholestearin and lecithin, and of other substances which are obviously to be regarded as excretory in character. It is necessary in the intestine for the complete absorption of the fats in normal amount, but even in its absence a considerable amount of fat can still be absorbed. The constituents which it contains in solution in largest quantity are the sodium salts of certain acids called the bile acids, and these bile salts are not excreted, but are reabsorbed, and undergo a circulation in the blood known as the circulation of the bile.

These few statements briefly summarise our experimental knowledge as to the action and physiological properties of the bile, and have given a basis to many theories.

It has been argued by some from the fact that bile contains no digestive enzyme, and from the presence in the fluid of certain constituents which are certainly excretory, that the bile is to be regarded purely as an excretion; but this view gives no explanation of the reabsorption of the bile salts, which are the most abundant constituent.